IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Linda S. Mansfield, Mary G. Rossano, Alice J. Murphy, and Ruth A. Vrable

Serial No.: 09/670,096 Group Art Unit: 1645

Filing Date: September 26, 2000

Title: VACCINE TO CONTROL EQUINE PROTOZOAL MYELOENCEPHALITIS IN HORSES

Examiner: Nina M. Minnifield

Mail Stop Appeal Brief - Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

BRIEF UNDER 37 C.F.R. § 1.192

Sir:

This is an appeal from a final rejection in the above entitled application. The claims on appeal are set forth as Appendix A. An oral hearing will be requested. Enclosed are three (3) copies of this Brief and the fee due upon filing of the Brief.

(1) Real Party in Interest

The real party in interest is the Board of Trustees operating Michigan State University, East Lansing, Michigan, a constitutional corporation of the

State of Michigan, which is the assignee of the above entitled application.

(2) Related Appeals and Interferences

The present application is a divisional application of Application Serial No. 09/513,086 ('086), filed February 24, 2000, and which claims benefit of a provisional patent Application No. 60/152,193, filed September 2, 1999. The '086 application relates to a vaccine comprising the 16 and 30 kDa antigens. The application is pending.

The present application is also related to Application Serial No. 09/670,096 ('096), which relates to an antibody vaccine comprising antibodies against the 16 ±4 and 30 ±4 kDa antigens; Application Serial No. 09/670,355 ('355), which relates to a vaccine comprising DNA encoding the 16 ±4 and 30 ±4 kDa antigens; Application Serial No. 09/669,833 ('833), which relates methods for making antibodies against the 16 ±4 and 30 ±4 kDa antigens; and Application Serial No. 09/670,244 ('224) which relates to recombinant protein comprising the 16 ±4 and 30 ±4 kDa antigens. The above applications were all filed on September 26, 2000. The '355 and '096 applications are on appeal. The remainder

of the applications are pending. No application has been allowed.

There are no other related appeals and interferences.

(3) Status of Claims

Claims 1, 2, and 21 are pending. No claims have been allowed.

(4) Status of Amendments

An Amendment After Final was filed April 22, 2003. The amendment was entered.

(5) Summary of Invention

As set forth in Claim 1, the applicants provide a composition for treating an equid infected with *Sarcocystis neurona* (Specification: page 15, lines 11-15) comprising a mixture of isolated antibodies against a 16 ±4 kDa antigen of *Sarcocystis neurona* and isolated antibodies against a 30 ±4 kDa antigen of *Sarcocystis neurona* (Specification: paragraph bridging pages 26-27) wherein the antibodies are from serum of an animal immunized with the antigen (Specification: page 26, lines 27-32; page 26, line 32, to page 27, line 22,

and Example 1) and wherein the mixture is in a pharmaceutically acceptable carrier (Specification: page 14, lines 1-31).

As set forth in Claim 2, the applicants provide a further embodiment of the composition in which the antibodies are monoclonal antibodies (Specification: page 27, lines 4-22, and Example 1).

As set forth in Claim 21, the applicants also provide a method for treating an equid infected with *Sarcocystis neurona* (Specification: page 15, lines 11-15) comprising (a) providing antibodies against a 16 ±4 kDa antigen and a 30 ±4 kDa antigen, both of which are specific to *Sarcocystis neurona*, wherein the antibodies are selected from the group consisting of polyclonal antibodies (Specification: page 26, lines 27-32; page 26, line 32, to page 27, line 3) and monoclonal antibodies (Specification: page 27, lines 4-22, and Example 1), wherein the antibodies are from serum from an animal immunized with the antigen (Specification: page 26, lines 27-32; page 26, line 32, to page 27, line 22, and Example 1), and wherein the antibodies are in a pharmaceutically acceptable carrier (Specification: page 14, lines 1-31); and (b) inoculating the equid with the mixture to treat the equid (Specification: page 13,

lines 24-35; page 15, lines 11-15).

(6) Issues

(A) Claims 1 and 2 remain rejected under 35 U.S.C. § 102(b) as being anticipated by Liang *et al.* (Infect. Immun. 66: 1834-1838 (1998)) because Liang teaches antiserum from horses infected with *Sarcocystis neurona* contain antibodies against the 16 and 30 kDa antigens.

(B) Claim 21 remains rejected under 35 U.S.C. § 112, first paragraph, as not being enabled by the specification because there are no working examples in the specification demonstrating any *in vivo* method of treating a horse with the claimed antibodies against the 16 and 30 kDa.

(7) Grouping of Claims

Claims 1 and 2 each stands or falls on its own. Claim 21 stands or falls on its own.

(8) Argument

(A) Claims 1 and 2 rejected under 35 U.S.C. § 102(b) as being anticipated by Liang *et al.*

Claims 1 and 2 are not believed to be

anticipated by Liang under 35 U.S.C. § 102(b). The applicants' claims claim compositions which comprise a mixture of isolated antibodies against the 16 and 30 kDa antigens in a pharmaceutically acceptable carrier and wherein the antibodies are from serum from an animal inoculated with the antigen. Claim 2 consists essentially of a mixture of monoclonal antibodies against the 16 and 30 kDa antigens.

Liang shows in Figure 1 that horses with EPM have serum containing antibodies against various combinations of the 11, 14, 16, and 30 kDa antigens. Some sera and CSF contain only antibodies against 16 and 30 kDa antigens whereas other sera also contains antibodies against the 11 and 14 kD antigens as well. While Liang's serum and CSF are filtered to remove infectious contaminants, the serum and CSF still contains a variety of other serum constituents such as antibodies against a variety of other pathogens. In contrast, in the applicants' currently claimed composition the antibodies are from serum from an animal inoculated with the antigens and the composition includes a pharmaceutically acceptable carrier. This is believed to distinghuish the applicants' claimed composition (Claims 1 or 2) from Liang's composition.

Furthermore, with respect to Claim 2 in particular, the applicants' claimed composition comprises monoclonal antibodies against the antigens. Serum and CSF from horses with EPM certainly do not contain monoclonal antibodies as set forth in Claim 2.Therefore, even if Claim 1 were found to be anticipated by the prior art, the monoclonal antibodies of Claim 2 clearly distinguish Claim 2 from the prior art.

In light of the above, the applicants' claimed composition as set forth in Claim 1 or Claim 2 is distinguishable from the serum and CSF of Liang and thus, not anticipated by Liang.

(B) Claim 21 was rejected under 35 U.S.C. § 112, first paragraph.

The applicants believe that the specification provides enablement which is commensurate with the scope of Claim 21.

Claim 21 provides a method for treating horses infected with *Sarcocystis neurona* by providing a mixture comprising antibodies against the 16 ±4 and 30 ±4 kDa antigens in a pharmaceutically acceptable carrier and inoculating the horse with the mixture. When all of the evidence relating to the factors set forth in M.P.E.P.

§ 2164.01(a) for determining whether a disclosure satisfies the enablement requirement is considered, the evidence as a whole shows that the scope of the applicants' presently amended claims is enabled by the applicants' disclosure.

The applicants' specification teaches that *Sarcocystis neurona* possesses a 16 ±4 and a 30 ±4 kDa antigen, both which are specific to *Sarcocystis neurona*, and which are useful for producing vaccines (Specification: page 13, lines 16-23). The applicants teach a variety of ways for vaccinating horses (Specification: page 13, lines 24-35), teach that the vaccines can comprise antibodies (Specification: page 15, lines 13-15), and teach how to make polyclonal antibodies (Specification: para. bridging pages 26-27) and monoclonal antibodies (Specification: page 27, lines 4-22; Example 1) for the vaccines. The applicants teach that antibody vaccines can be used for therapeutic treatment of horses infected with *Sarcocystis neurona* (Specification: page 15, lines 11-15) and to provide passive immunity (Specification: page 26, lines 20-26).

Liang (1998) provides an idea of what the state of the art was at about the time of the applicants' invention. Liang (1998) teaches that

antisera from horses with EPM contain antibodies against 11, 14, 16, and 30 kDa antigens. Liang (1998) also teaches that *Sarcocystis neurona* "was sensitive to specific antibodies but that "a 10-min exposure to antiserum was required to yield significant reduction in parasite production" (Liang (1998): page 1837, right col.). Liang (1998) further teaches that protective antibodies against some apicomplexan parasites may be effective *in vitro* but ineffective *in vivo* which Liang (1998) cites Hines *et al.* (Inf. Immun. 63: 349-352 (1995); copy enclosed) for support.

Hines showed that immunizing cattle with a vaccine containing the MSA-1 antigen of *Babesia bovis* failed to protect the immunized cattle against challenge with *Babesia bovis*. However, Hines suggested that an efficacious vaccine would include at least a second antigen of *Babesia bovis* as was shown in the case of a malaria vaccine which contained two antigens and which was shown to be effective *in vivo* against the malaria parasite whereas a vaccine containing only one of the antigens was partially effective (Hines: page 351, second para.). The applicants' claimed composition contains antibodies against a second antigen.

Liang (1998) also teaches that "[t]he high

rate of exposure [of horses] to *Sarcocystis neurona* and the relatively low incidence of clinical EPM indicate that most horses develop effective immunity that may prevent entry into the central nervous system [citing various sources]" (Liang (1998): page 1834, right col.). Liang (1998) also teaches that antisera from horses with EPM have antibodies against several *Sarcocystis neurona* antigens, in particular the 16 and 30 kDa antigens. Liang (1998) identifies the 11, 14, 16, and 30 as major immunogens (Liang (1998): Figure 1). Liang (1998) further teaches that antibodies against the 30 kDa antigen are not recognized as specific (Liang (1998): page 1837, left col., first para.), which suggests that the 30 kDa antigen is common to all *Sarcocystis* spp. and not unique to *Sarcocystis neurona*. However, the applicants teach that antisera from horses infected with *Sarcocystis neurona* contain antibodies specific for the 30 kDa antigen of *Sarcocystis neurona*. Therefore, since many horses exposed to *Sarcocystis neurona* do not have clinical signs of EPM but have immunity to *Sarcocystis neurona*, Liang (1998) teaches that the major immunogens of *Sarcocystis neurona* infected horses comprise the 16 and 30 kDa antigens, and the applicants teach that horses make antibodies specific to the 30 ±4 kDa

antigen, there is a nexus between the 16 and 30 kDa-specific antibodies identified in Liang and by the applicants and a composition comprising the 16 ±4 and 30 ±4 kDa antigens for treating horses infected with *Sarcocystis neurona*.

Liang (1998) provides further support for a nexus between the 16 ±4 and 30 ±4 kDa-specific antibodies and their use in a composition for treating horses infected with *Sarcocystis neurona*. Liang (1998) teaches that the 14, 16, and 30 kDa antigens are surface antigens (Liang (1998): page 1836, left col., and Figure 3). Because surface antigens are generally important in the function or life-cycle of the organism, it is reasonable to expect that blocking the activity of one or more surface antigens by binding the antigens with antibodies would interrupt the function or life-cycle of the *Sarcocystis neurona*. Therefore, the applicants' presently claimed composition, which would bind to the 16 ±4 and 30 ±4 kDa antigens, would be expected to have at least some efficacy in treating horses infected with *Sarcocystis neurona*.

While Liang (1998) teaches that a "10-minute exposure to antiserum was required to yield a significant reduction in parasite production" and that

"may partially explain why protective antibodies to some apicomplexan parasites are effective in vitro but not in vivo" (Liang (1998): page 1837, left col., third para.), Liang (1998) suggests that the reason is that "newly released parasites are exposed to serum for a shorter time in vivo, and the access of neutralization-sensitive epitopes to antibody may be limited" and that "[m]erozoites in vivo may move more directly from cell to cell" (Liang (1998): page 1837, left col., third para.). While the statements suggest that humoral responses to *Sarcocystis neurona* may be of limited efficacy in inhibiting parasite production, the statements do not suggest that humoral responses would have no efficacy against disease caused by *Sarcocystis neurona*. In fact, Liang (1998) suggests that antibodies against the 14 and 16 kDa antigens may be efficacious against the EPM disease caused by *Sarcocystis neurona* because Liang (1998) also teaches that "in the case of EPM, disease occurs only after the merozoite passes through the vascular endothelium of the blood-brain barrier into the central nervous system, and so humoral responses may play an essential role in blocking this migration" (Liang (1998): page 1837, left col., third para.) particularly since "specific cytotoxic T-cells

are ineffective in attacking merozoites migrating to the central nervous system in the bloodstream" (Liang (1998): page 1837, left col., third para.). Liang (1998) further suggests that the 14 and 16 kDa antigens may be useful components of a subunit vaccine. Thus, the above suggests that the applicants' claimed composition comprising antibodies against the 16 ±4 and 30 ±4 kDa antigens might provide an efficacious means for treating horses infected with *Sarcocystis neurona*.

Therefore, in light of the above, the state of the art reasonably suggests that the applicants' claimed composition comprising a mixture of antibodies against several *Sarcocystis neurona* antigens would likely be efficacious for treating horses infected with *Sarcocystis neurona*. While the efficacy of a composition comprising a single antibody against a single antigen might be unpredictable, in light of the state of the art, one skilled in the art would likely predict that a composition comprising a mixture of antibodies against several antigens (16 ±4 and 30 ±4 kDa antigens) would provide an efficacious treatment for horses infected with *Sarcocystis neurona*.

Further, while the applicants do not provide working examples showing that their claimed composition

provides an efficacious treatment for horses infected with *Sarcocystis neurona*, the applicants' disclosure in light of the state of the art suggests that the applicants' claimed composition would be useful for treating horses infected with *Sarcocystis neurona*. The only experimentation expected would be adjusting the amounts of each antibody in the composition. Such experimentation is routine and would be performed by one skilled in the art even if the applicants had provided working examples.

Furthermroe, as taught in Liang, most horses exposed to *Sarcocystis neurona* do not develop clinical EPM which suggests that the horses have developed effective immunity which may have prevented entry of the parasite into the central nervous system (CNS). Thus, it appears that horses with EPM have some defect in their immune response which allowed the parasite to enter the CNS.

Liang also teaches that the immunodominant antigens are the 11, 14, 16, and 30 kDa antigens. Liang further provides data which suggests that while antibodies against the 14 and 16 kDa antigens are neutralizing, antibodies against the 30 kDa antigen are not neutralizing.

In contrast to the data in Liang, the applicants have data which show that at least some antibodies against the 30 kDa antigen are neutralizing. The data are presented in a Declaration under 37 C.F.R. § 1.132 (Appendix B) which clearly shows not only that some CSF antibodies against the 30 kDa antigen are neutralizing but that CSF containing antibodies against both the 16 and the 30 kDa antigens appeared to be more neutralizing than either antibody species alone. The applicants' data shows that a composition containing antibodies against both the 16 and 30 kDa antigens would be reasonably expected to provide an effective treatment for horses infected with *Sarcocystis neurona*. The applicants' data shows that if one skilled in the art had relied upon the teachings of Liang for guidance, they would have mistakenly believed that antibodies against the 30 kDa antigen were non-neutralizing.

It is important to note that in Liang, serum and CSF samples were obtained from horses with a clinical diagnosis of a neurologic disorder resembling EPM. As taught by the applicants, horses with lameness or other neurologic diseases are being misdiagnosed as having EPM (page 4, lines 16-17). Since Liang does not demonstrate that the horses with clinical signs

resembling EPM were actually infected with *Sarcocystis neurona* it is not known whether any of the Liang samples reported to contain only antibodies against a 30 kDa antigen (Liang: Figure 2) were infected with *Sarcocystis neurona*. The horses might have been infected with another *Sarcocystis* species which induces an antibody that reacts non-specifically with the 30 kDa antigen from *Sarcocystis neurona*. For example, the applicants show in U.S. Patent No. 6,153,394 to Mansfield *et al.*, which had been incorporated by reference on page 13, lines 16-20, that the 16 and 30 kDa antigens are *Sarcocystis neurona*-specific (Mansfield: col. 7, lines 37-46; Figure 4), that serum from horses known not to be infected with *Sarcocystis neurona* contain antibodies which in Western blots appear to cross-react with the 16 and 30 kDa antigens (Mansfield: col. 6, lines 43-50; Figure 3), and that the observed antibody cross-reactivity might be because antibodies to other apicomplexian species can occur at or near the about 12 and 29 kDa bands and therefore may cause false-positive test results (Mansfield: col. 3, lines 11-19). Thus, Liang's support for the statement that antibodies against the 30 kDa antigen are not neutralizing is equivocal at best. Therefore, Liang is

not believed to provide suitable support for the rejection.

Nevertheless, in light of the applicants' disclosure and declaration and other teachings in Liang, it would appear to be reasonable to believe that horses with EPM have an inadequate immune response to the parasite which is not sufficient to prevent entry of the parasite into the CNS and that boosting the immune response with antibodies against the 16 and 30 kDa antigens might provide a sufficient boost to an infected horse's immune response to inhibit entry of the parasite into the CSF. The applicants' currently claimed method provides such a means for treating such infected horses. The applicants' composition would boost the concentration of antibodies against the 16 and 30 kDa antigens in the horse. It would be reasonable to expect that the increased level of antibodies against those two antigens would have a beneficial effect on the horse such as preventing further entry of the parasite into the CNS even if the increased level of antibodies did not cure the horse of the parasite.

Therefore, in light of the above, Claim 21 is believed to be enabled by the applicants' disclosure.

(9) Conclusion

As shown above, Claims 1 and Claim 2 are each separately patentable over the prior art and Claim 21 is enabled by the specification. Remand to the Examiner for Notice of Allowance is requested.

Respectfully,

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Attachments: Appendix A - Claims on Appeal
Appendix B - Declaration Under 37 CFR 1.132

APPENDIX A

-1-

A composition for treating an equid infected with *Sarcocystis neurona* comprising a mixture of isolated antibodies against a 16 ±4 kDa antigen of *Sarcocystis neurona* and isolated antibodies against a 30 ±4 kDa antigen of *Sarcocystis neurona* wherein the antibodies are from serum of an animal immunized with the antigen and wherein the mixture is in a pharmaceutically acceptable carrier.

-2-

The composition of Claim 1 wherein the antibodies are monoclonal antibodies.

A method for treating an equid infected with *Sarcocystis neurona* comprising:

(a) providing antibodies against a 16 ±4 kDa antigen and a 30 ±4 kDa antigen, both of which are specific to *Sarcocystis neurona*, wherein the antibodies are selected from the group consisting of polyclonal antibodies and monoclonal antibodies, wherein the antibodies are from serum from an animal immunized with the antigen, and wherein the antibodies are in a pharmaceutically acceptable carrier; and

(b) inoculating the equid with the mixture to treat the equid.

APPENDIX B

MSU 4.1-526
Appl. No. 09/670,096
April 1, 2003
Reply to Office Action of Jan. 23, 2003

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Linda S. Mansfield, Mary G. Rossano, Alice J. Murphy, and Ruth A. Vrable

Serial No. 09/670,096 Group Art Unit: 1645

Filing Date: 2000 September 26

Title: VACCINE TO CONTROL EQUINE PROTOZOAL MYELOENCEPHALITIS IN HORSES

Examiner: Padmavathi Basker, Ph.D.

BOX AF

Commissioner of Patents and Trademarks

Washington, D.C. 20231

DECLARATION UNDER 37 C.F.R. § 1.132

Dear sir:

Alice J. Murphy states as follows.

(1) That she is an inventor of the invention in the above entitled application.

(2) That she performed an experiment in East Lansing, Michigan at Michigan State University (assignee of the present invention) to determine the neutralizing ability of antibodies against the 16 and 30 kDa antigens. The results showed that cerebral spinal fluid (CSF) from horses infected with *Sarcocystis neurona* which contained only antibodies that were strongly reacting against the 30 kDa antigen was neutralizing as was CSF which contained only antibodies that were strongly reacting against the 16 kDa antigen.

(3) That the experiment used CSF samples isolated from three horses infected with *Sarcocystis neurona*. CSF from the first infected horse contained antibodies which strongly

reacted against both the 16 kDa and 30 kDa antigens, CSF from the second infected horse contained antibodies which strongly reacted against only the 30 kDa antigen, and CFS from the third infected horse contained antibodies which strongly reacted against only the 16 kDa antigen. The controls for the experiment consisted of CSF from a horse from India known not to be infected with *Sarcocystis neurona* and Tris-buffered saline (TBS) containing 5% fetal bovine serum (FBS). The first horse was also culture positive for *Sarcocystis neurona*. Neural tissue from the horse at necropsy was ground up and inoculated into the media on equine dermal cells in culture. The media was replaced after 24, 48, and possibly 72 hours post inoculation and then weekly thereafter. The first plaque was seen on day 29 after inoculation. The merozoites from the plaques were subsequently identified as *Sarcocystis neurona* by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) techniques. Horses 2 and 3 had clinical signs which suggested the horses were infected with *Sarcocystis neurona*.

(4) That the experiment was performed as follows.

(a) Merozoites of *Sarcocystis neurona* from culture were washed in Tris-buffered saline (TBS) twice to remove media. The merozoites had been previously obtained from neural tissue from a Michigan horse infected with *Sarcocystis neurona*. The identity of the merozoites had been confirmed by PCR and RFLP.

(b) The washed merozoites were diluted in TBS and 35 μL was added to each of the 6 tubes comprising each of the horse groups. To test viability of the merozoites, 35 μL of the merozoites were affixed to a slide by cytospin (two replicates) and stained. There appeared to be about 20 to 30 viable merozoites per 35 μL. The stained cytospin provided an idea of the number of normal appearing and potentially viable merozoites per 35 μL aliquot. To confirm the viability of the merozoites, a real viability test was performed as follows. 70 μL of the merozoites were added directly to a 25 mL flask of confluent equine dermal cells. An additional 70 μL of merozoites were washed and spun twice in the same manner as the test samples. The pellet was

suspended in media and divided between two 25 mL flasks of confluent equine dermal cells. Two hours after inoculating the flasks, moving (spinning as they do when they are "drilling" into a cell) merozoites were seen in all three flasks. In addition, all three flasks developed plaques. Plaques consisted of a minimum of three rounded-up cells contiguous with one another to a maximum of a bare area of surface surrounded by rounded-up cells. (Cells round up when infected and come loose off the well or flask when the cell is heavily laden with parasite or the cell bursts from the parasite load. Since infective merozoites tend to move only into neighboring cells (unless one shakes up the flask which happens when the media is changed), bare areas surrounded by rounded up cells in older plaques are seen). The test for real viability confirmed that the merozoites used in the experiment described herein were viable.

(c) The CSF sample from horse 1 was diluted 1:10, 1:20, 1:40, 1:80, 1:160 with TBS and the ~~CFS~~ CSF samples from horses 2 and 3 were diluted 1:10, 1:20, 1:40, 1:80 with TBS. 200 μL of undiluted CSF and each dilution of CSF was each added to a tube of merozoites. The controls consisted of undiluted Indian horse CSF and TBS containing 5% FBS. There were six replicates of each of the samples and controls.

(d) All the samples and controls were incubated for one hour at 37° C.

(e) Each tube was centrifuged for 4 minutes at 1000 xg to pellet the merozoites. The supernatant fraction was removed and the merozoites were washed by resuspending the merozoite pellets in 300 μL TBS and centrifuging to pellet the merozoites and removing the supernatant fraction. Two washes were performed.

(f) After the final wash, the merozoite pellets were each resuspended in 200 μL of media and then each suspension was added to a well of a six–well plate of a monolayer of equine dermal cells which was just confluent.

(g) The plates were gently swirled to distribute the merozoites over the

monolayer and the cells then incubated at 37° C in a 5% CO_2 atmosphere. The media was replaced after 24 hours and then replaced weekly thereafter.

(h) Any plaques which formed were counted at five and six weeks post inoculation.

(5) That the results of the experiment are shown in Table 1; that the results show that the CSF containing antibodies against either antigen was separately neutralizing when used undiluted compared to the controls; that the results further show that CSF containing both antibodies was neutralizing even when used at a 1:10 dilution; and, that the results show that the neutralizing ability of the undiluted CSF from all three infected horses appears to be significant as was the 1:10 dilution of the CSF from the first horse.

Table 1

Sample	Dilution	Mean No. Plaques (SE) at 5 weeks post inoculation	Mean No. Plaques (SE) at 6 weeks post inoculation
1	undiluted	**4.3 (0.5)**	**20.0 (1.2)**
(Anti-16 & -30)	1:10	**3.2 (0.6)**	**30.0 (1.8)**
	1:20	6.0 (0.6)	55.5 (2.7)
	1:40	7.2 (0.4)	54.2 (2.8)
	1:80	9.0 (0.6)	52.7 (1.7)
	1:160	8.8 (0.4)	54.5 (2.3)
2	undiluted	**3.7 (0.4)**	**37.5 (2.4)**
(Anti-30)	1:10	7.8 (0.3)	57.5 (2.5)
	1:20	6.8 (0.3)	53.2 (3.0)
	1:40	8.3 (0.8)	53.2 (1.7)
	1:80	9.8 (0.4)	57.8 (3.1)
3	undiluted	**4.0 (0.6)**	**36.3 (2.1)**
(Anti-16)	1:10	7.7 (0.8)	55.3 (2.5)
	1:20	8.7 (0.6)	58.3 (3.1)
	1:40	8.7 (0.9)	55.5 (2.6)
	1:80	7.7 (0.8)	49.2 (2.4)
Indian horse	undiluted	8.7 (0.4)	56.8 (2.7)
5% FBS	undiluted	8.5 (0.6)	54.8 (3.2)

(6) That the results of Liang *et al.*, published in Infection and Immunity 66: 1834-1838 (1998), which shows that antibodies against the 30 kDa antigen in serum or CSF from horses infected with *Sarcocystis neurona* are not neutralizing, are not consistent with the results described herein and are not believed to be correct.

(7) That the undersigned declares further that all statements made herein of her own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such wilful false statements may jeopardize the validity of the application or any patent issuing thereon.

MSU 4.1-526
Appl. No. 09/670,096
April 1, 2003
Reply to Office Action of Jan. 23, 2003

Alice J. Murphy

Date: 4-17-03

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The present application is also related to Application Serial No. 09/670,096 ('096), which relates to an antibody vaccine comprising antibodies against the 16 ±4 and 30 ±4 kDa antigens; Application Serial No. 09/670,355 ('355), which relates to a vaccine comprising DNA encoding the 16 ±4 and 30 ±4 kDa antigens; Application Serial No. 09/669,833 ('833), which relates methods for making antibodies against the 16 ±4 and 30 ±4 kDa antigens; and Application Serial No. 09/670,244 ('224) which relates to recombinant protein comprising the 16 ±4 and 30 ±4 kDa antigens. The above applications were all filed on September 26, 2000. The '355 and '096 applications are on appeal. The remainder

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and Example 1) and wherein the mixture is in a pharmaceutically acceptable carrier (Specification: page 14, lines 1-31).

As set forth in Claim 2, the applicants provide a further embodiment of the composition in which the antibodies are monoclonal antibodies (Specification: page 27, lines 4-22, and Example 1).

As set forth in Claim 21, the applicants also provide a method for treating an equid infected with *Sarcocystis neurona* (Specification: page 15, lines 11-15) comprising (a) providing antibodies against a 16 ±4 kDa antigen and a 30 ±4 kDa antigen, both of which are specific to *Sarcocystis neurona*, wherein the antibodies are selected from the group consisting of polyclonal antibodies (Specification: page 26, lines 27-32; page 26, line 32, to page 27, line 3) and monoclonal antibodies (Specification: page 27, lines 4-22, and Example 1), wherein the antibodies are from serum from an animal immunized with the antigen (Specification: page 26, lines 27-32; page 26, line 32, to page 27, line 22, and Example 1), and wherein the antibodies are in a pharmaceutically acceptable carrier (Specification: page 14, lines 1-31); and (b) inoculating the equid with the mixture to treat the equid (Specification: page 13,

lines 24-35; page 15, lines 11-15).

(6) Issues

(A) Claims 1 and 2 remain rejected under 35 U.S.C. § 102(b) as being anticipated by Liang *et al.* (Infect. Immun. 66: 1834-1838 (1998)) because Liang teaches antiserum from horses infected with *Sarcocystis neurona* contain antibodies against the 16 and 30 kDa antigens.

(B) Claim 21 remains rejected under 35 U.S.C. § 112, first paragraph, as not being enabled by the specification because there are no working examples in the specification demonstrating any *in vivo* method of treating a horse with the claimed antibodies against the 16 and 30 kDa.

(7) Grouping of Claims

Claims 1 and 2 each stands or falls on its own. Claim 21 stands or falls on its own.

(8) Argument

(A) Claims 1 and 2 rejected under 35 U.S.C. § 102(b) as being anticipated by Liang *et al.*

Claims 1 and 2 are not believed to be

anticipated by Liang under 35 U.S.C. § 102(b). The applicants' claims claim compositions which comprise a mixture of isolated antibodies against the 16 and 30 kDa antigens in a pharmaceutically acceptable carrier and wherein the antibodies are from serum from an animal inoculated with the antigen. Claim 2 consists essentially of a mixture of monoclonal antibodies against the 16 and 30 kDa antigens.

Liang shows in Figure 1 that horses with EPM have serum containing antibodies against various combinations of the 11, 14, 16, and 30 kDa antigens. Some sera and CSF contain only antibodies against 16 and 30 kDa antigens whereas other sera also contains antibodies against the 11 and 14 kD antigens as well. While Liang's serum and CSF are filtered to remove infectious contaminants, the serum and CSF still contains a variety of other serum constituents such as antibodies against a variety of other pathogens. In contrast, in the applicants' currently claimed composition the antibodies are from serum from an animal inoculated with the antigens and the composition includes a pharmaceutically acceptable carrier. This is believed to distinghuish the applicants' claimed composition (Claims 1 or 2) from Liang's composition.

Furthermore, with respect to Claim 2 in particular, the applicants' claimed composition comprises monoclonal antibodies against the antigens. Serum and CSF from horses with EPM certainly do not contain monoclonal antibodies as set forth in Claim 2.Therefore, even if Claim 1 were found to be anticipated by the prior art, the monoclonal antibodies of Claim 2 clearly distinguish Claim 2 from the prior art.

In light of the above, the applicants' claimed composition as set forth in Claim 1 or Claim 2 is distinguishable from the serum and CSF of Liang and thus, not anticipated by Liang.

(B) Claim 21 was rejected under 35 U.S.C. § 112, first paragraph.

The applicants believe that the specification provides enablement which is commensurate with the scope of Claim 21.

Claim 21 provides a method for treating horses infected with *Sarcocystis neurona* by providing a mixture comprising antibodies against the 16 ±4 and 30 ±4 kDa antigens in a pharmaceutically acceptable carrier and inoculating the horse with the mixture. When all of the evidence relating to the factors set forth in M.P.E.P.

§ 2164.01(a) for determining whether a disclosure satisfies the enablement requirement is considered, the evidence as a whole shows that the scope of the applicants' presently amended claims is enabled by the applicants' disclosure.

The applicants' specification teaches that *Sarcocystis neurona* possesses a 16 ±4 and a 30 ±4 kDa antigen, both which are specific to *Sarcocystis neurona*, and which are useful for producing vaccines (Specification: page 13, lines 16-23). The applicants teach a variety of ways for vaccinating horses (Specification: page 13, lines 24-35), teach that the vaccines can comprise antibodies (Specification: page 15, lines 13-15), and teach how to make polyclonal antibodies (Specification: para. bridging pages 26-27) and monoclonal antibodies (Specification: page 27, lines 4-22; Example 1) for the vaccines. The applicants teach that antibody vaccines can be used for therapeutic treatment of horses infected with *Sarcocystis neurona* (Specification: page 15, lines 11-15) and to provide passive immunity (Specification: page 26, lines 20-26).

Liang (1998) provides an idea of what the state of the art was at about the time of the applicants' invention. Liang (1998) teaches that

antisera from horses with EPM contain antibodies against 11, 14, 16, and 30 kDa antigens. Liang (1998) also teaches that *Sarcocystis neurona* "was sensitive to specific antibodies but that "a 10-min exposure to antiserum was required to yield significant reduction in parasite production" (Liang (1998): page 1837, right col.). Liang (1998) further teaches that protective antibodies against some apicomplexan parasites may be effective *in vitro* but ineffective *in vivo* which Liang (1998) cites Hines *et al*. (Inf. Immun. 63: 349-352 (1995); copy enclosed) for support.

Hines showed that immunizing cattle with a vaccine containing the MSA-1 antigen of *Babesia bovis* failed to protect the immunized cattle against challenge with *Babesia bovis*. However, Hines suggested that an efficacious vaccine would include at least a second antigen of *Babesia bovis* as was shown in the case of a malaria vaccine which contained two antigens and which was shown to be effective *in vivo* against the malaria parasite whereas a vaccine containing only one of the antigens was partially effective (Hines: page 351, second para.). The applicants' claimed composition contains antibodies against a second antigen.

Liang (1998) also teaches that "[t]he high

rate of exposure [of horses] to *Sarcocystis neurona* and the relatively low incidence of clinical EPM indicate that most horses develop effective immunity that may prevent entry into the central nervous system [citing various sources]" (Liang (1998): page 1834, right col.). Liang (1998) also teaches that antisera from horses with EPM have antibodies against several *Sarcocystis neurona* antigens, in particular the 16 and 30 kDa antigens. Liang (1998) identifies the 11, 14, 16, and 30 as major immunogens (Liang (1998): Figure 1). Liang (1998) further teaches that antibodies against the 30 kDa antigen are not recognized as specific (Liang (1998): page 1837, left col., first para.), which suggests that the 30 kDa antigen is common to all *Sarcocystis* spp. and not unique to *Sarcocystis neurona*. However, the applicants teach that antisera from horses infected with *Sarcocystis neurona* contain antibodies specific for the 30 kDa antigen of *Sarcocystis neurona*. Therefore, since many horses exposed to *Sarcocystis neurona* do not have clinical signs of EPM but have immunity to *Sarcocystis neurona*, Liang (1998) teaches that the major immunogens of *Sarcocystis neurona* infected horses comprise the 16 and 30 kDa antigens, and the applicants teach that horses make antibodies specific to the 30 ±4 kDa

antigen, there is a nexus between the 16 and 30 kDa-specific antibodies identified in Liang and by the applicants and a composition comprising the 16 ±4 and 30 ±4 kDa antigens for treating horses infected with *Sarcocystis neurona*.

Liang (1998) provides further support for a nexus between the 16 ±4 and 30 ±4 kDa-specific antibodies and their use in a composition for treating horses infected with *Sarcocystis neurona*. Liang (1998) teaches that the 14, 16, and 30 kDa antigens are surface antigens (Liang (1998): page 1836, left col., and Figure 3). Because surface antigens are generally important in the function or life-cycle of the organism, it is reasonable to expect that blocking the activity of one or more surface antigens by binding the antigens with antibodies would interrupt the function or life-cycle of the *Sarcocystis neurona*. Therefore, the applicants' presently claimed composition, which would bind to the 16 ±4 and 30 ±4 kDa antigens, would be expected to have at least some efficacy in treating horses infected with *Sarcocystis neurona*.

While Liang (1998) teaches that a "10-minute exposure to antiserum was required to yield a significant reduction in parasite production" and that

"may partially explain why protective antibodies to some apicomplexan parasites are effective in vitro but not in vivo" (Liang (1998): page 1837, left col., third para.), Liang (1998) suggests that the reason is that "newly released parasites are exposed to serum for a shorter time in vivo, and the access of neutralization-sensitive epitopes to antibody may be limited" and that "[m]erozoites in vivo may move more directly from cell to cell" (Liang (1998): page 1837, left col., third para.). While the statements suggest that humoral responses to *Sarcocystis neurona* may be of limited efficacy in inhibiting parasite production, the statements do not suggest that humoral responses would have no efficacy against disease caused by *Sarcocystis neurona*. In fact, Liang (1998) suggests that antibodies against the 14 and 16 kDa antigens may be efficacious against the EPM disease caused by *Sarcocystis neurona* because Liang (1998) also teaches that "in the case of EPM, disease occurs only after the merozoite passes through the vascular endothelium of the blood-brain barrier into the central nervous system, and so humoral responses may play an essential role in blocking this migration" (Liang (1998): page 1837, left col., third para.) particularly since "specific cytotoxic T-cells

are ineffective in attacking merozoites migrating to the central nervous system in the bloodstream" (Liang (1998): page 1837, left col., third para.). Liang (1998) further suggests that the 14 and 16 kDa antigens may be useful components of a subunit vaccine. Thus, the above suggests that the applicants' claimed composition comprising antibodies against the 16 ±4 and 30 ±4 kDa antigens might provide an efficacious means for treating horses infected with *Sarcocystis neurona*.

Therefore, in light of the above, the state of the art reasonably suggests that the applicants' claimed composition comprising a mixture of antibodies against several *Sarcocystis neurona* antigens would likely be efficacious for treating horses infected with *Sarcocystis neurona*. While the efficacy of a composition comprising a single antibody against a single antigen might be unpredictable, in light of the state of the art, one skilled in the art would likely predict that a composition comprising a mixture of antibodies against several antigens (16 ±4 and 30 ±4 kDa antigens) would provide an efficacious treatment for horses infected with *Sarcocystis neurona*.

Further, while the applicants do not provide working examples showing that their claimed composition

provides an efficacious treatment for horses infected with *Sarcocystis neurona*, the applicants' disclosure in light of the state of the art suggests that the applicants' claimed composition would be useful for treating horses infected with *Sarcocystis neurona*. The only experimentation expected would be adjusting the amounts of each antibody in the composition. Such experimentation is routine and would be performed by one skilled in the art even if the applicants had provided working examples.

Furthermroe, as taught in Liang, most horses exposed to *Sarcocystis neurona* do not develop clinical EPM which suggests that the horses have developed effective immunity which may have prevented entry of the parasite into the central nervous system (CNS). Thus, it appears that horses with EPM have some defect in their immune response which allowed the parasite to enter the CNS.

Liang also teaches that the immunodominant antigens are the 11, 14, 16, and 30 kDa antigens. Liang further provides data which suggests that while antibodies against the 14 and 16 kDa antigens are neutralizing, antibodies against the 30 kDa antigen are not neutralizing.

In contrast to the data in Liang, the applicants have data which show that at least some antibodies against the 30 kDa antigen are neutralizing. The data are presented in a Declaration under 37 C.F.R. § 1.132 (Appendix B) which clearly shows not only that some CSF antibodies against the 30 kDa antigen are neutralizing but that CSF containing antibodies against both the 16 and the 30 kDa antigens appeared to be more neutralizing than either antibody species alone. The applicants' data shows that a composition containing antibodies against both the 16 and 30 kDa antigens would be reasonably expected to provide an effective treatment for horses infected with *Sarcocystis neurona*. The applicants' data shows that if one skilled in the art had relied upon the teachings of Liang for guidance, they would have mistakenly believed that antibodies against the 30 kDa antigen were non-neutralizing.

It is important to note that in Liang, serum and CSF samples were obtained from horses with a clinical diagnosis of a neurologic disorder resembling EPM. As taught by the applicants, horses with lameness or other neurologic diseases are being misdiagnosed as having EPM (page 4, lines 16-17). Since Liang does not demonstrate that the horses with clinical signs

resembling EPM were actually infected with *Sarcocystis neurona* it is not known whether any of the Liang samples reported to contain only antibodies against a 30 kDa antigen (Liang: Figure 2) were infected with *Sarcocystis neurona*. The horses might have been infected with another *Sarcocystis* species which induces an antibody that reacts non-specifically with the 30 kDa antigen from *Sarcocystis neurona*. For example, the applicants show in U.S. Patent No. 6,153,394 to Mansfield *et al.*, which had been incorporated by reference on page 13, lines 16-20, that the 16 and 30 kDa antigens are *Sarcocystis neurona*-specific (Mansfield: col. 7, lines 37-46; Figure 4), that serum from horses known not to be infected with *Sarcocystis neurona* contain antibodies which in Western blots appear to cross-react with the 16 and 30 kDa antigens (Mansfield: col. 6, lines 43-50; Figure 3), and that the observed antibody cross-reactivity might be because antibodies to other apicomplexian species can occur at or near the about 12 and 29 kDa bands and therefore may cause false-positive test results (Mansfield: col. 3, lines 11-19). Thus, Liang's support for the statement that antibodies against the 30 kDa antigen are not neutralizing is equivocal at best. Therefore, Liang is

not believed to provide suitable support for the rejection.

Nevertheless, in light of the applicants' disclosure and declaration and other teachings in Liang, it would appear to be reasonable to believe that horses with EPM have an inadequate immune response to the parasite which is not sufficient to prevent entry of the parasite into the CNS and that boosting the immune response with antibodies against the 16 and 30 kDa antigens might provide a sufficient boost to an infected horse's immune response to inhibit entry of the parasite into the CSF. The applicants' currently claimed method provides such a means for treating such infected horses. The applicants' composition would boost the concentration of antibodies against the 16 and 30 kDa antigens in the horse. It would be reasonable to expect that the increased level of antibodies against those two antigens would have a beneficial effect on the horse such as preventing further entry of the parasite into the CNS even if the increased level of antibodies did not cure the horse of the parasite.

Therefore, in light of the above, Claim 21 is believed to be enabled by the applicants' disclosure.

(9) Conclusion

As shown above, Claims 1 and Claim 2 are each separately patentable over the prior art and Claim 21 is enabled by the specification. Remand to the Examiner for Notice of Allowance is requested.

Respectfully,

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Attachments: Appendix A - Claims on Appeal
Appendix B - Declaration Under 37 CFR 1.132

APPENDIX A

-1-

A composition for treating an equid infected with *Sarcocystis neurona* comprising a mixture of isolated antibodies against a 16 ±4 kDa antigen of *Sarcocystis neurona* and isolated antibodies against a 30 ±4 kDa antigen of *Sarcocystis neurona* wherein the antibodies are from serum of an animal immunized with the antigen and wherein the mixture is in a pharmaceutically acceptable carrier.

-2-

The composition of Claim 1 wherein the antibodies are monoclonal antibodies.

A method for treating an equid infected with *Sarcocystis neurona* comprising:

(a) providing antibodies against a 16 ±4 kDa antigen and a 30 ±4 kDa antigen, both of which are specific to *Sarcocystis neurona*, wherein the antibodies are selected from the group consisting of polyclonal antibodies and monoclonal antibodies, wherein the antibodies are from serum from an animal immunized with the antigen, and wherein the antibodies are in a pharmaceutically acceptable carrier; and

(b) inoculating the equid with the mixture to treat the equid.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Linda S. Mansfield, Mary G. Rossano, Alice J. Murphy, and Ruth A. Vrable

Serial No.: 09/670,096 Group Art Unit: 1645

Filing Date: September 26, 2000

Title: VACCINE TO CONTROL EQUINE PROTOZOAL MYELOENCEPHALITIS IN HORSES

Examiner: Nina M. Minnifield

Mail Stop Appeal Brief - Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

BRIEF UNDER 37 C.F.R. § 1.192

Sir:

This is an appeal from a final rejection in the above entitled application. The claims on appeal are set forth as Appendix A. An oral hearing will be requested. Enclosed are three (3) copies of this Brief and the fee due upon filing of the Brief.

(1) Real Party in Interest

The real party in interest is the Board of Trustees operating Michigan State University, East Lansing, Michigan, a constitutional corporation of the

State of Michigan, which is the assignee of the above entitled application.

(2) Related Appeals and Interferences

The present application is a divisional application of Application Serial No. 09/513,086 ('086), filed February 24, 2000, and which claims benefit of a provisional patent Application No. 60/152,193, filed September 2, 1999. The '086 application relates to a vaccine comprising the 16 and 30 kDa antigens. The application is pending.

The present application is also related to Application Serial No. 09/670,096 ('096), which relates to an antibody vaccine comprising antibodies against the 16 ±4 and 30 ±4 kDa antigens; Application Serial No. 09/670,355 ('355), which relates to a vaccine comprising DNA encoding the 16 ±4 and 30 ±4 kDa antigens; Application Serial No. 09/669,833 ('833), which relates methods for making antibodies against the 16 ±4 and 30 ±4 kDa antigens; and Application Serial No. 09/670,244 ('224) which relates to recombinant protein comprising the 16 ±4 and 30 ±4 kDa antigens. The above applications were all filed on September 26, 2000. The '355 and '096 applications are on appeal. The remainder

of the applications are pending. No application has been allowed.

There are no other related appeals and interferences.

(3) Status of Claims

Claims 1, 2, and 21 are pending. No claims have been allowed.

(4) Status of Amendments

An Amendment After Final was filed April 22, 2003. The amendment was entered.

(5) Summary of Invention

As set forth in Claim 1, the applicants provide a composition for treating an equid infected with *Sarcocystis neurona* (Specification: page 15, lines 11-15) comprising a mixture of isolated antibodies against a 16 ±4 kDa antigen of *Sarcocystis neurona* and isolated antibodies against a 30 ±4 kDa antigen of *Sarcocystis neurona* (Specification: paragraph bridging pages 26-27) wherein the antibodies are from serum of an animal immunized with the antigen (Specification: page 26, lines 27-32; page 26, line 32, to page 27, line 22,

and Example 1) and wherein the mixture is in a pharmaceutically acceptable carrier (Specification: page 14, lines 1-31).

As set forth in Claim 2, the applicants provide a further embodiment of the composition in which the antibodies are monoclonal antibodies (Specification: page 27, lines 4-22, and Example 1).

As set forth in Claim 21, the applicants also provide a method for treating an equid infected with *Sarcocystis neurona* (Specification: page 15, lines 11-15) comprising (a) providing antibodies against a 16 ±4 kDa antigen and a 30 ±4 kDa antigen, both of which are specific to *Sarcocystis neurona*, wherein the antibodies are selected from the group consisting of polyclonal antibodies (Specification: page 26, lines 27-32; page 26, line 32, to page 27, line 3) and monoclonal antibodies (Specification: page 27, lines 4-22, and Example 1), wherein the antibodies are from serum from an animal immunized with the antigen (Specification: page 26, lines 27-32; page 26, line 32, to page 27, line 22, and Example 1), and wherein the antibodies are in a pharmaceutically acceptable carrier (Specification: page 14, lines 1-31); and (b) inoculating the equid with the mixture to treat the equid (Specification: page 13,

lines 24-35; page 15, lines 11-15).

(6) Issues

(A) Claims 1 and 2 remain rejected under 35 U.S.C. § 102(b) as being anticipated by Liang *et al.* (Infect. Immun. 66: 1834-1838 (1998)) because Liang teaches antiserum from horses infected with *Sarcocystis neurona* contain antibodies against the 16 and 30 kDa antigens.

(B) Claim 21 remains rejected under 35 U.S.C. § 112, first paragraph, as not being enabled by the specification because there are no working examples in the specification demonstrating any *in vivo* method of treating a horse with the claimed antibodies against the 16 and 30 kDa.

(7) Grouping of Claims

Claims 1 and 2 each stands or falls on its own. Claim 21 stands or falls on its own.

(8) Argument

(A) Claims 1 and 2 rejected under 35 U.S.C. § 102(b) as being anticipated by Liang *et al.*

Claims 1 and 2 are not believed to be

anticipated by Liang under 35 U.S.C. § 102(b). The applicants' claims claim compositions which comprise a mixture of isolated antibodies against the 16 and 30 kDa antigens in a pharmaceutically acceptable carrier and wherein the antibodies are from serum from an animal inoculated with the antigen. Claim 2 consists essentially of a mixture of monoclonal antibodies against the 16 and 30 kDa antigens.

Liang shows in Figure 1 that horses with EPM have serum containing antibodies against various combinations of the 11, 14, 16, and 30 kDa antigens. Some sera and CSF contain only antibodies against 16 and 30 kDa antigens whereas other sera also contains antibodies against the 11 and 14 kD antigens as well. While Liang's serum and CSF are filtered to remove infectious contaminants, the serum and CSF still contains a variety of other serum constituents such as antibodies against a variety of other pathogens. In contrast, in the applicants' currently claimed composition the antibodies are from serum from an animal inoculated with the antigens and the composition includes a pharmaceutically acceptable carrier. This is believed to distinghuish the applicants' claimed composition (Claims 1 or 2) from Liang's composition.

Furthermore, with respect to Claim 2 in particular, the applicants' claimed composition comprises monoclonal antibodies against the antigens. Serum and CSF from horses with EPM certainly do not contain monoclonal antibodies as set forth in Claim 2.Therefore, even if Claim 1 were found to be anticipated by the prior art, the monoclonal antibodies of Claim 2 clearly distinguish Claim 2 from the prior art.

In light of the above, the applicants' claimed composition as set forth in Claim 1 or Claim 2 is distinguishable from the serum and CSF of Liang and thus, not anticipated by Liang.

(B) Claim 21 was rejected under 35 U.S.C. § 112, first paragraph.

The applicants believe that the specification provides enablement which is commensurate with the scope of Claim 21.

Claim 21 provides a method for treating horses infected with *Sarcocystis neurona* by providing a mixture comprising antibodies against the 16 ±4 and 30 ±4 kDa antigens in a pharmaceutically acceptable carrier and inoculating the horse with the mixture. When all of the evidence relating to the factors set forth in M.P.E.P.

§ 2164.01(a) for determining whether a disclosure satisfies the enablement requirement is considered, the evidence as a whole shows that the scope of the applicants' presently amended claims is enabled by the applicants' disclosure.

The applicants' specification teaches that *Sarcocystis neurona* possesses a 16 ±4 and a 30 ±4 kDa antigen, both which are specific to *Sarcocystis neurona*, and which are useful for producing vaccines (Specification: page 13, lines 16-23). The applicants teach a variety of ways for vaccinating horses (Specification: page 13, lines 24-35), teach that the vaccines can comprise antibodies (Specification: page 15, lines 13-15), and teach how to make polyclonal antibodies (Specification: para. bridging pages 26-27) and monoclonal antibodies (Specification: page 27, lines 4-22; Example 1) for the vaccines. The applicants teach that antibody vaccines can be used for therapeutic treatment of horses infected with *Sarcocystis neurona* (Specification: page 15, lines 11-15) and to provide passive immunity (Specification: page 26, lines 20-26).

Liang (1998) provides an idea of what the state of the art was at about the time of the applicants' invention. Liang (1998) teaches that

antisera from horses with EPM contain antibodies against 11, 14, 16, and 30 kDa antigens. Liang (1998) also teaches that *Sarcocystis neurona* "was sensitive to specific antibodies but that "a 10-min exposure to antiserum was required to yield significant reduction in parasite production" (Liang (1998): page 1837, right col.). Liang (1998) further teaches that protective antibodies against some apicomplexan parasites may be effective *in vitro* but ineffective *in vivo* which Liang (1998) cites Hines *et al.* (Inf. Immun. 63: 349-352 (1995); copy enclosed) for support.

Hines showed that immunizing cattle with a vaccine containing the MSA-1 antigen of *Babesia bovis* failed to protect the immunized cattle against challenge with *Babesia bovis*. However, Hines suggested that an efficacious vaccine would include at least a second antigen of *Babesia bovis* as was shown in the case of a malaria vaccine which contained two antigens and which was shown to be effective *in vivo* against the malaria parasite whereas a vaccine containing only one of the antigens was partially effective (Hines: page 351, second para.). The applicants' claimed composition contains antibodies against a second antigen.

Liang (1998) also teaches that "[t]he high

rate of exposure [of horses] to *Sarcocystis neurona* and the relatively low incidence of clinical EPM indicate that most horses develop effective immunity that may prevent entry into the central nervous system [citing various sources]" (Liang (1998): page 1834, right col.). Liang (1998) also teaches that antisera from horses with EPM have antibodies against several *Sarcocystis neurona* antigens, in particular the 16 and 30 kDa antigens. Liang (1998) identifies the 11, 14, 16, and 30 as major immunogens (Liang (1998): Figure 1). Liang (1998) further teaches that antibodies against the 30 kDa antigen are not recognized as specific (Liang (1998): page 1837, left col., first para.), which suggests that the 30 kDa antigen is common to all *Sarcocystis* spp. and not unique to *Sarcocystis neurona*. However, the applicants teach that antisera from horses infected with *Sarcocystis neurona* contain antibodies specific for the 30 kDa antigen of *Sarcocystis neurona*. Therefore, since many horses exposed to *Sarcocystis neurona* do not have clinical signs of EPM but have immunity to *Sarcocystis neurona*, Liang (1998) teaches that the major immunogens of *Sarcocystis neurona* infected horses comprise the 16 and 30 kDa antigens, and the applicants teach that horses make antibodies specific to the 30 ±4 kDa

antigen, there is a nexus between the 16 and 30 kDa-specific antibodies identified in Liang and by the applicants and a composition comprising the 16 ±4 and 30 ±4 kDa antigens for treating horses infected with *Sarcocystis neurona*.

Liang (1998) provides further support for a nexus between the 16 ±4 and 30 ±4 kDa-specific antibodies and their use in a composition for treating horses infected with *Sarcocystis neurona*. Liang (1998) teaches that the 14, 16, and 30 kDa antigens are surface antigens (Liang (1998): page 1836, left col., and Figure 3). Because surface antigens are generally important in the function or life-cycle of the organism, it is reasonable to expect that blocking the activity of one or more surface antigens by binding the antigens with antibodies would interrupt the function or life-cycle of the *Sarcocystis neurona*. Therefore, the applicants' presently claimed composition, which would bind to the 16 ±4 and 30 ±4 kDa antigens, would be expected to have at least some efficacy in treating horses infected with *Sarcocystis neurona*.

While Liang (1998) teaches that a "10-minute exposure to antiserum was required to yield a significant reduction in parasite production" and that

"may partially explain why protective antibodies to some apicomplexan parasites are effective in vitro but not in vivo" (Liang (1998): page 1837, left col., third para.), Liang (1998) suggests that the reason is that "newly released parasites are exposed to serum for a shorter time in vivo, and the access of neutralization-sensitive epitopes to antibody may be limited" and that "[m]erozoites in vivo may move more directly from cell to cell" (Liang (1998): page 1837, left col., third para.). While the statements suggest that humoral responses to *Sarcocystis neurona* may be of limited efficacy in inhibiting parasite production, the statements do not suggest that humoral responses would have no efficacy against disease caused by *Sarcocystis neurona*. In fact, Liang (1998) suggests that antibodies against the 14 and 16 kDa antigens may be efficacious against the EPM disease caused by *Sarcocystis neurona* because Liang (1998) also teaches that "in the case of EPM, disease occurs only after the merozoite passes through the vascular endothelium of the blood-brain barrier into the central nervous system, and so humoral responses may play an essential role in blocking this migration" (Liang (1998): page 1837, left col., third para.) particularly since "specific cytotoxic T-cells

are ineffective in attacking merozoites migrating to the central nervous system in the bloodstream" (Liang (1998): page 1837, left col., third para.). Liang (1998) further suggests that the 14 and 16 kDa antigens may be useful components of a subunit vaccine. Thus, the above suggests that the applicants' claimed composition comprising antibodies against the 16 ±4 and 30 ±4 kDa antigens might provide an efficacious means for treating horses infected with *Sarcocystis neurona*.

Therefore, in light of the above, the state of the art reasonably suggests that the applicants' claimed composition comprising a mixture of antibodies against several *Sarcocystis neurona* antigens would likely be efficacious for treating horses infected with *Sarcocystis neurona*. While the efficacy of a composition comprising a single antibody against a single antigen might be unpredictable, in light of the state of the art, one skilled in the art would likely predict that a composition comprising a mixture of antibodies against several antigens (16 ±4 and 30 ±4 kDa antigens) would provide an efficacious treatment for horses infected with *Sarcocystis neurona*.

Further, while the applicants do not provide working examples showing that their claimed composition

provides an efficacious treatment for horses infected with *Sarcocystis neurona*, the applicants' disclosure in light of the state of the art suggests that the applicants' claimed composition would be useful for treating horses infected with *Sarcocystis neurona*. The only experimentation expected would be adjusting the amounts of each antibody in the composition. Such experimentation is routine and would be performed by one skilled in the art even if the applicants had provided working examples.

Furthermroe, as taught in Liang, most horses exposed to *Sarcocystis neurona* do not develop clinical EPM which suggests that the horses have developed effective immunity which may have prevented entry of the parasite into the central nervous system (CNS). Thus, it appears that horses with EPM have some defect in their immune response which allowed the parasite to enter the CNS.

Liang also teaches that the immunodominant antigens are the 11, 14, 16, and 30 kDa antigens. Liang further provides data which suggests that while antibodies against the 14 and 16 kDa antigens are neutralizing, antibodies against the 30 kDa antigen are not neutralizing.

In contrast to the data in Liang, the applicants have data which show that at least some antibodies against the 30 kDa antigen are neutralizing. The data are presented in a Declaration under 37 C.F.R. § 1.132 (Appendix B) which clearly shows not only that some CSF antibodies against the 30 kDa antigen are neutralizing but that CSF containing antibodies against both the 16 and the 30 kDa antigens appeared to be more neutralizing than either antibody species alone. The applicants' data shows that a composition containing antibodies against both the 16 and 30 kDa antigens would be reasonably expected to provide an effective treatment for horses infected with *Sarcocystis neurona*. The applicants' data shows that if one skilled in the art had relied upon the teachings of Liang for guidance, they would have mistakenly believed that antibodies against the 30 kDa antigen were non-neutralizing.

It is important to note that in Liang, serum and CSF samples were obtained from horses with a clinical diagnosis of a neurologic disorder resembling EPM. As taught by the applicants, horses with lameness or other neurologic diseases are being misdiagnosed as having EPM (page 4, lines 16-17). Since Liang does not demonstrate that the horses with clinical signs

resembling EPM were actually infected with *Sarcocystis neurona* it is not known whether any of the Liang samples reported to contain only antibodies against a 30 kDa antigen (Liang: Figure 2) were infected with *Sarcocystis neurona*. The horses might have been infected with another *Sarcocystis* species which induces an antibody that reacts non-specifically with the 30 kDa antigen from *Sarcocystis neurona*. For example, the applicants show in U.S. Patent No. 6,153,394 to Mansfield *et al.*, which had been incorporated by reference on page 13, lines 16-20, that the 16 and 30 kDa antigens are *Sarcocystis neurona*-specific (Mansfield: col. 7, lines 37-46; Figure 4), that serum from horses known not to be infected with *Sarcocystis neurona* contain antibodies which in Western blots appear to cross-react with the 16 and 30 kDa antigens (Mansfield: col. 6, lines 43-50; Figure 3), and that the observed antibody cross-reactivity might be because antibodies to other apicomplexian species can occur at or near the about 12 and 29 kDa bands and therefore may cause false-positive test results (Mansfield: col. 3, lines 11-19). Thus, Liang's support for the statement that antibodies against the 30 kDa antigen are not neutralizing is equivocal at best. Therefore, Liang is

not believed to provide suitable support for the rejection.

Nevertheless, in light of the applicants' disclosure and declaration and other teachings in Liang, it would appear to be reasonable to believe that horses with EPM have an inadequate immune response to the parasite which is not sufficient to prevent entry of the parasite into the CNS and that boosting the immune response with antibodies against the 16 and 30 kDa antigens might provide a sufficient boost to an infected horse's immune response to inhibit entry of the parasite into the CSF. The applicants' currently claimed method provides such a means for treating such infected horses. The applicants' composition would boost the concentration of antibodies against the 16 and 30 kDa antigens in the horse. It would be reasonable to expect that the increased level of antibodies against those two antigens would have a beneficial effect on the horse such as preventing further entry of the parasite into the CNS even if the increased level of antibodies did not cure the horse of the parasite.

Therefore, in light of the above, Claim 21 is believed to be enabled by the applicants' disclosure.

(9) Conclusion

As shown above, Claims 1 and Claim 2 are each separately patentable over the prior art and Claim 21 is enabled by the specification. Remand to the Examiner for Notice of Allowance is requested.

Respectfully,

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Attachments: Appendix A - Claims on Appeal
Appendix B - Declaration Under 37 CFR 1.132

APPENDIX A

-1-

A composition for treating an equid infected with *Sarcocystis neurona* comprising a mixture of isolated antibodies against a 16 ±4 kDa antigen of *Sarcocystis neurona* and isolated antibodies against a 30 ±4 kDa antigen of *Sarcocystis neurona* wherein the antibodies are from serum of an animal immunized with the antigen and wherein the mixture is in a pharmaceutically acceptable carrier.

-2-

The composition of Claim 1 wherein the antibodies are monoclonal antibodies.

A method for treating an equid infected with *Sarcocystis neurona* comprising:

(a) providing antibodies against a 16 ±4 kDa antigen and a 30 ±4 kDa antigen, both of which are specific to *Sarcocystis neurona*, wherein the antibodies are selected from the group consisting of polyclonal antibodies and monoclonal antibodies, wherein the antibodies are from serum from an animal immunized with the antigen, and wherein the antibodies are in a pharmaceutically acceptable carrier; and

(b) inoculating the equid with the mixture to treat the equid.

APPENDIX B

MSU 4.1-526
Appl. No. 09/670,096
April 1, 2003
Reply to Office Action of Jan. 23, 2003

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Linda S. Mansfield, Mary G. Rossano, Alice J. Murphy, and Ruth A. Vrable

Serial No. 09/670,096 Group Art Unit: 1645

Filing Date: 2000 September 26

Title: VACCINE TO CONTROL EQUINE PROTOZOAL MYELOENCEPHALITIS IN HORSES

Examiner: Padmavathi Basker, Ph.D.

BOX AF

Commissioner of Patents and Trademarks

Washington, D.C. 20231

DECLARATION UNDER 37 C.F.R. § 1.132

Dear sir:

Alice J. Murphy states as follows.

(1) That she is an inventor of the invention in the above entitled application.

(2) That she performed an experiment in East Lansing, Michigan at Michigan State University (assignee of the present invention) to determine the neutralizing ability of antibodies against the 16 and 30 kDa antigens. The results showed that cerebral spinal fluid (CSF) from horses infected with *Sarcocystis neurona* which contained only antibodies that were strongly reacting against the 30 kDa antigen was neutralizing as was CSF which contained only antibodies that were strongly reacting against the 16 kDa antigen.

(3) That the experiment used CSF samples isolated from three horses infected with *Sarcocystis neurona*. CSF from the first infected horse contained antibodies which strongly

reacted against both the 16 kDa and 30 kDa antigens, CSF from the second infected horse contained antibodies which strongly reacted against only the 30 kDa antigen, and CFS from the third infected horse contained antibodies which strongly reacted against only the 16 kDa antigen. The controls for the experiment consisted of CSF from a horse from India known not to be infected with *Sarcocystis neurona* and Tris-buffered saline (TBS) containing 5% fetal bovine serum (FBS). The first horse was also culture positive for *Sarcocystis neurona*. Neural tissue from the horse at necropsy was ground up and inoculated into the media on equine dermal cells in culture. The media was replaced after 24, 48, and possibly 72 hours post inoculation and then weekly thereafter. The first plaque was seen on day 29 after inoculation. The merozoites from the plaques were subsequently identified as *Sarcocystis neurona* by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) techniques. Horses 2 and 3 had clinical signs which suggested the horses were infected with *Sarcocystis neurona*.

(4) That the experiment was performed as follows.

(a) Merozoites of *Sarcocystis neurona* from culture were washed in Tris-buffered saline (TBS) twice to remove media. The merozoites had been previously obtained from neural tissue from a Michigan horse infected with *Sarcocystis neurona*. The identity of the merozoites had been confirmed by PCR and RFLP.

(b) The washed merozoites were diluted in TBS and 35 μL was added to each of the 6 tubes comprising each of the horse groups. To test viability of the merozoites, 35 μL of the merozoites were affixed to a slide by cytospin (two replicates) and stained. There appeared to be about 20 to 30 viable merozoites per 35 μL. The stained cytospin provided an idea of the number of normal appearing and potentially viable merozoites per 35 μL aliquot. To confirm the viability of the merozoites, a real viability test was performed as follows. 70 μL of the merozoites were added directly to a 25 mL flask of confluent equine dermal cells. An additional 70 μL of merozoites were washed and spun twice in the same manner as the test samples. The pellet was

suspended in media and divided between two 25 mL flasks of confluent equine dermal cells. Two hours after inoculating the flasks, moving (spinning as they do when they are "drilling" into a cell) merozoites were seen in all three flasks. In addition, all three flasks developed plaques. Plaques consisted of a minimum of three rounded-up cells contiguous with one another to a maximum of a bare area of surface surrounded by rounded-up cells. (Cells round up when infected and come loose off the well or flask when the cell is heavily laden with parasite or the cell bursts from the parasite load. Since infective merozoites tend to move only into neighboring cells (unless one shakes up the flask which happens when the media is changed), bare areas surrounded by rounded up cells in older plaques are seen). The test for real viability confirmed that the merozoites used in the experiment described herein were viable.

(c) The CSF sample from horse 1 was diluted 1:10, 1:20, 1:40, 1:80, 1:160 with TBS and the ~~CFS~~ CSF samples from horses 2 and 3 were diluted 1:10, 1:20, 1:40, 1:80 with TBS. 200 μL of undiluted CSF and each dilution of CSF was each added to a tube of merozoites. The controls consisted of undiluted Indian horse CSF and TBS containing 5% FBS. There were six replicates of each of the samples and controls.

(d) All the samples and controls were incubated for one hour at 37° C.

(e) Each tube was centrifuged for 4 minutes at 1000 xg to pellet the merozoites. The supernatant fraction was removed and the merozoites were washed by resuspending the merozoite pellets in 300 μL TBS and centrifuging to pellet the merozoites and removing the supernatant fraction. Two washes were performed.

(f) After the final wash, the merozoite pellets were each resuspended in 200 μL of media and then each suspension was added to a well of a six–well plate of a monolayer of equine dermal cells which was just confluent.

(g) The plates were gently swirled to distribute the merozoites over the

monolayer and the cells then incubated at 37° C in a 5% CO_2 atmosphere. The media was replaced after 24 hours and then replaced weekly thereafter.

(h) Any plaques which formed were counted at five and six weeks post inoculation.

(5) That the results of the experiment are shown in Table 1; that the results show that the CSF containing antibodies against either antigen was separately neutralizing when used undiluted compared to the controls; that the results further show that CSF containing both antibodies was neutralizing even when used at a 1:10 dilution; and, that the results show that the neutralizing ability of the undiluted CSF from all three infected horses appears to be significant as was the 1:10 dilution of the CSF from the first horse.

Table 1

Sample	Dilution	Mean No. Plaques (SE) at 5 weeks post inoculation	Mean No. Plaques (SE) at 6 weeks post inoculation
1	undiluted	**4.3 (0.5)**	**20.0 (1.2)**
(Anti-16 & -30)	1:10	**3.2 (0.6)**	**30.0 (1.8)**
	1:20	6.0 (0.6)	55.5 (2.7)
	1:40	7.2 (0.4)	54.2 (2.8)
	1:80	9.0 (0.6)	52.7 (1.7)
	1:160	8.8 (0.4)	54.5 (2.3)
2	undiluted	**3.7 (0.4)**	**37.5 (2.4)**
(Anti-30)	1:10	7.8 (0.3)	57.5 (2.5)
	1:20	6.8 (0.3)	53.2 (3.0)
	1:40	8.3 (0.8)	53.2 (1.7)
	1:80	9.8 (0.4)	57.8 (3.1)
3	undiluted	**4.0 (0.6)**	**36.3 (2.1)**
(Anti-16)	1:10	7.7 (0.8)	55.3 (2.5)
	1:20	8.7 (0.6)	58.3 (3.1)
	1:40	8.7 (0.9)	55.5 (2.6)
	1:80	7.7 (0.8)	49.2 (2.4)
Indian horse	undiluted	8.7 (0.4)	56.8 (2.7)
5% FBS	undiluted	8.5 (0.6)	54.8 (3.2)

(6) That the results of Liang *et al.*, published in Infection and Immunity 66: 1834-1838 (1998), which shows that antibodies against the 30 kDa antigen in serum or CSF from horses infected with *Sarcocystis neurona* are not neutralizing, are not consistent with the results described herein and are not believed to be correct.

(7) That the undersigned declares further that all statements made herein of her own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such wilful false statements may jeopardize the validity of the application or any patent issuing thereon.

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Alice J. Murphy

Date: 4-17-03